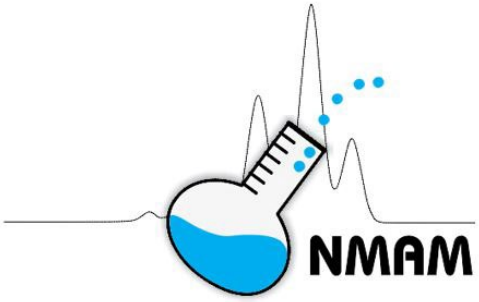

NIOSH Manual of Analytical Methods (NMAM), 5th Edition

Measurement and Characterization of Fibrous Particles in Workplace Atmospheres

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1 Version 2 updates

NMAM Chapter FI is based upon a previous version written by Paul A. Baron [NIOSH 2003] that was adapted from a review article [Baron 2001]. The following are in this 2022 revision:

- Chapter title was updated
- References were updated
- Introduction was updated
- Adoption of elongate mineral particle terminology and made congruent with NIOSH Current Intelligence Bulletin 62, *Asbestos Fibers and Other Elongate Mineral Particles: State of the Science and Roadmap for Research* [NIOSH 2011]
- Information on the effects of over-grinding was added to the Sample Preparation Section within the Polarized Light Microscopy (PLM) of Bulk Materials Section
- Information on using commercially available equipment for fiber classification was added to Fiber Separation Section
- Additional information on removing long fibers in air flow streams was added to the Fiber Separation Section
- Other techniques were moved from a subsection within the Polarized Light Microscopy (PLM) of Bulk Materials Section to a separate section with expanded information on identification

2 Introduction

Disease from exposure to fibrous particles in workplace atmospheres has provided much of the impetus for regulations governing such exposures. Exposure to fibrous minerals, such as asbestos, nonasbestos minerals, and some zeolites, where the material is insoluble *in vivo*, can cascade in a number of biological events that lead to disease [Aust et al. 2011; EPA 2020; Khaliullin et al. 2020; Lippman 2014; Mazurek et al. 2017; Nielson et al. 2014; NIOSH 2011]. Workers also face possible exposures to other commercially introduced synthetic fibers, such as mineral wools, ceramic, carbon nanotubes (CNT), and carbon nanofibers (CNF) [Lippman 2014; NIOSH 2013]. The concern with biopersistent synthetic fibrous materials is that they can produce similar biological responses as asbestos, however, epidemiological studies indicate that they may be less hazardous than asbestos [Lippman 2014]. This is an active area of research.

Natural organic fibers have been shown to not produce the long-term health effects of some of the inorganic fibers unless they are industrially processed and contain harmful additives [Jarvholm 2000]. Natural cellulose can be processed to produce several fibrous types, such as bacterial cellulose (BC), microcrystalline cellulose (MC), microfibrillated cellulose (MFC), and cellulose nanocrystals (CNCs). These materials are gaining wide use in industry [Camarero-Espinosa et al. 2016]. Strategies for risk assessment have been laid out for cellulose nanomaterials [Shatkin and Kim 2015], and an update was published stating that conclusions cannot yet be made about any long-term health consequences [Ede et al. 2019].

The sections of this chapter cover fiber dimension considerations, NIOSH methods for phase contrast microscopy (PCM), polarized light microscopy (PLM), electron microscopy (EM), optical detection, fiber separation, and other developing techniques, followed by conclusions. The chapter is not meant to be a comprehensive review of these subjects but rather an introduction with extensive references for further reading and education.

a. What is a fiber?

The NIOSH Bulletin 62 on *Asbestos Fibers and Other Elongate Mineral Particles: State of the Science and Roadmap for Research* [NIOSH 2011] has provided a much-needed framework for terminology related to fibrous particles. The reader is encouraged to review this "roadmap" for much greater detail into these topics.

Common English dictionaries define a "fiber" as "a long threadlike substance or material." Unfortunately, the term "fiber" has been used inconsistently and in widely varying contexts in scientific literature, which has led to ambiguous exposure-response relationships and exposure measurements. The roadmap addresses these issues with inconsistent terminology [NIOSH 2011]. Note that the terminology used in this chapter is consistent with the roadmap.

An elongate particle (EP) is any particle (of any material) having an aspect ratio greater than 3:1. This definition is desirable because fibers are subdivided into many categories, which may not be easily determined or well defined. For example, a single mineral particle or fiber may be in a transition state and can vary in characteristics from one portion of itself to another. These subcategories become very important in defining asbestos. The definition for asbestos as an elongate mineral particle (EMP) follows [NIOSH 1990, 2011]:

> An acicular single crystal or similarly elongate polycrystalline aggregate particles. Such particles have macroscopic properties, such as flexibility, high aspect ratio, silky luster, and axial lineation. These particles have attained their shape primarily because of manifold dislocation planes that are randomly oriented in two axes but parallel in the third. *Note:* Upon microscopic examination, only particles that have a 3:1 or greater aspect ratio are defined as fibers. Other macroscopic properties used to define fibers cannot be ascertained for individual particles examined microscopically.

NIOSH, using these definitions, and specifically EMP, issued an updated recommended exposure limit (REL) and a clarification [NIOSH 2011]:

- a *countable elongate mineral particle* (EMP) is any fiber or fragment of a mineral longer than 5 µm with a minimum aspect ratio of 3:1 when viewed microscopically with use of NIOSH Analytical Method 7400 ('A' rules) or its equivalent; and

- a *covered mineral* is any mineral having the crystal structure and elemental composition of one of the asbestos varieties (chrysotile, riebeckite asbestos [crocidolite], cummingtonite-grunerite asbestos [amosite], anthophyllite asbestos, tremolite asbestos, and actinolite asbestos) or one of their nonasbestiform analogs (the serpentine minerals antigorite and lizardite, and the amphibole minerals contained in the cummingtonite-grunerite mineral series, the tremolite-ferroactinolite mineral series, and the glaucophane-riebeckite mineral series).

This clarification of the NIOSH REL for airborne asbestos fibers and related EMPs results in *no change* in counts made, as defined by NIOSH Method 7400 ("A" rules) [NIOSH 2019]. However, it clarifies definitionally that EMPs included in the count are not necessarily asbestos fibers.

Asbestos and asbestiform will be discussed further in the Asbestos Section.

b. Mineral and nonmineral fibers

The term "mineral fiber" has been frequently used by nonmineralogists to describe EMPs found in the thoracic cavity (sometimes referred to as thoracic EMPs), originating either from an asbestiform habit (e.g., as asbestos fibers) or a nonasbestiform habit (e.g., as needle-like [acicular] or prismatic crystals), as well as EMPs that result from the crushing or fracturing of nonfibrous minerals (e.g., cleavage fragments). "Asbestiform" is a term applied to minerals with a macroscopic habit similar to that of asbestos. The lack of precision in these terms and the difficulty in translating macroscopic properties to microscopically identifiable characteristics contribute to miscommunication and uncertainty in identifying toxicity associated with various forms of minerals. Deposits may have more than one mineral habit and transitional minerals may be present, which make it difficult to describe the mineralogy clearly. EPs also come in a wide variety of nonmineral types from organic (plant and animal), synthetic such as polymer, glass, carbonaceous fibers, boron nitride fibers, etc.

c. Nonasbestos fibers with known health effects

There are "fibers of concern," not covered by asbestos policies, where there have been documented health effects. Erionite is perhaps the most worrisome example [NTP 2004]. An epidemic of malignant mesothelioma affecting several villages in Central Turkey has been studied for several decades [Baris and Grandjean 2006; Baris et al. 1981]. Homes and other buildings in those villages were traditionally constructed of blocks of local volcanic stone containing erionite, a fibrous zeolite mineral. A recently published prospective mortality study has documented that mesothelioma accounts for over 40% of deaths among those residing in the affected villages. Although no clear epidemic of erionite-caused disease has been documented elsewhere, the mineral occurs in the intermountain west of the United States, and a

recent publication reports a case of erionite-associated malignant mesothelioma in North America [Kliment et al. 2009].

On the basis of studies in rats, palygorskite (attapulgite) fibers longer than 5 micrometer (μm) were determined to possibly be carcinogenic to humans (Group 2B) [IARC 1997]. In experimental animals, the evidence was limited for the carcinogenicity of long sepiolite fiber (>5 μm) and inadequate to assess carcinogenicity of nonerionite fibrous zeolites (including clinoptilolite, mordenite, and phillipsite) and wollastonite (Group 3) [IARC 1997]. These Group 3 determinations highlight the need for additional research on nonasbestiform EMPs.

Occupational health interest in EMPs other than asbestos fibers has been focused primarily on fibrous minerals exploited commercially (e.g., wollastonite, sepiolite, and attapulgite) and mineral commodities that contain (e.g., Libby vermiculite) or may contain (e.g., upstate New York talc) asbestiform minerals [NIOSH 2014; Van Gosen 2007a]. Exposure to airborne thoracic EMPs generated from the crushing and fracturing of nonasbestiform amphibole minerals has also garnered substantial interest. The asbestos minerals, as well as other types of asbestiform minerals, are typically associated with other minerals in geologic formations at various locations in the United States [Van Gosen 2007b]. The biological significance of occupational exposure to airborne particles remains unknown for some of these minerals and can be difficult to ascertain given the mixed and sporadic nature of exposure in many work environments and the general lack of well-characterized exposure information.

There are man-made EPs of concern as well. Particularly important examples are CNTs and CNFs. NIOSH issued a REL for these of 1 microgram per cubic meter (1 $\mu g/m^3$) [NIOSH 2013]. These EPs have been shown in animal studies to produce inflammation in the lungs similar to crocidolite asbestos, migrate to the intrapleural space to produce mesothelial lesions [Xu et al. 2012], instigate systemic responses including an increase in inflammatory mediators in the blood, oxidant stress in aortic tissue, and increase plaque formation in an atherosclerotic mouse model [Erdely et al. 2009; Li J. et al. 2007, Li Z. et al. 2007]. CNTs and CNFs can be encountered in facilities ranging from research laboratories and production plants to operations where CNT and CNF are processed, used, disposed, or recycled. The findings of these studies and others indicate the need to limit worker exposure to CNTs and CNFs [NIOSH 2013].

d. Asbestos

"Asbestos" is a term used for certain minerals that have crystallized in a particular macroscopic habit (asbestiform) with certain commercially useful properties. The fibers of all varieties of asbestos are long, thin, and usually flexible when separated. One variety of asbestos, chrysotile, is a mineral in the serpentine group of sheet silicates. Chrysotile fibers consist of aggregates of long, thin, flexible fibrils that resemble scrolls or cylinders. The dimensions of individual chrysotile fibers depend on the extent to which the material has been manipulated. Five varieties of asbestos

are minerals in the amphibole group of double-chain silicates—riebeckite asbestos (crocidolite), cummingtonite-grunerite asbestos (amosite), anthophyllite asbestos, tremolite asbestos, and actinolite asbestos [Virta 2002].

These amphibole varieties occur in an "asbestiform" habit, though not all "asbestiform" minerals are asbestos. "Asbestos" and "asbestiform" are two commonly used terms that lack mineralogical precision. When certain minerals were marketed or regulated as asbestos, the mineral names had definitions that might have been imprecise at the time and might have changed over time. In particular, the mineral name "amosite" was a commercial term for a mineral that was not well defined at first. The definitions of amosite in the Dictionary of Mining, Mineral, and Related Terms [U.S. Bureau of Mines 1996] and in the Glossary of Geology [American Geological Institute 2005] allow for the possibility that amosite might be anthophyllite asbestos, although it is now known to be a mineral in the cummingtonite-grunerite series. This is one source of confusion in the literature.

A further source of confusion comes from the use of the geological terms for a mineral habit. Minerals of the same chemistry, differing only in the expression of their crystallinity (e.g., massive, fibrous, asbestiform, or prismatic), are not differentiated in geology as independent species. Thus, tremolite in an asbestiform crystal habit is not given a separate name (either chemical or common) from tremolite in a massive habit. It has been suggested that crystals grown in an "asbestiform" habit can be distinguished by certain characteristics. Examples of this are parallel or radiating growth of very thin and elongate crystals that are to some degree flexible, the presence of bundles of fibrils, and, for amphiboles, a particular combination of twinning, stacking faults, and defects [Chisholm 1973].

The geological conditions necessary for the formation of asbestiform crystals are not as common as those that produce other crystal habits. These other habits may occur without any accompanying asbestiform crystals. However, amphibole asbestos may also include additional amphiboles that, if separated, are not asbestiform [Brown and Gunter 2003]. The mineralogical community uses many terms, including fibril, fiber, fibrous, acicular, needlelike, prismatic, and columnar, to denote crystals that are elongate. In contrast, in sedimentology, similar terms have been more narrowly defined with specific axial ratios. Thus, it is not clear, even from a single reference source, exactly what range of morphologies are described by these terms and the degree of overlap, if any. For example, the Dictionary of Mining, Mineral, and Related Terms defines fibril as "a single fiber, which cannot be separated into smaller components without losing its fibrous properties or appearance," but also defines a fiber as "the smallest single strand of asbestos or other fibrous material" [U.S. Bureau of Mines 1996].

Asbestos has been the EMP type most associated with adverse health effects and disease for some time [Henderson and Leigh 2011], with many reviews [ATSDR 1990, 2001; Craighead and Gibbs 2008; Dement 1990; Institute of Medicine 2006; Rajhans and Sullivan 1981; Santee and Lott 2003; Selikoff and Lee 1978; Weill et al. 2018;

WHO 1986, 2012, 2014]. The three primary diseases associated with asbestos EMP exposure are asbestosis, the result of inflammation and collagen formation in lung tissue; lung cancer; and mesothelioma, a rare cancer of the linings of the internal organs, mostly seen in the lung lining, although it may also occur in the tissues lining the abdominal cavity and its organs. EMPs have demonstrated similar health effects to asbestos [Castranova et al. 1994; Lippman 2014; NIOSH 2011]. Determination of the toxicity of EMPs of nonasbestiform varieties, such as cleavage fragments, is an ongoing area of research [IARC 2012, 2017; Khaliullin 2020; Manning et al. 2002].

e. Toxicity aspects of fibers

A current theory describing the toxicity of EPs indicates that dimension, dose, surface chemistry, and biodurability in lung fluid are the four primary factors determining EP toxicity [Ishida et al. 2019; Lippmann 1990, 2014, 2020]. The first thing to consider is how EPs are deposited in the lungs. This depends on all the indicated parameters in Figure 1 in a complex fashion including aerodynamic properties. Both EP diameter and length are important in the deposition of EPs in the lungs and how long they are likely to remain in the lungs. EP diameter affects aerodynamic behavior, which favors small diameter EPs, to travel and deposit into the airways of the lungs and the gas exchange regions. However, larger diameter particles are affected more by gravitational settling, impaction, and interception, resulting in greater deposition further up in the respiratory tract. The saddle points, or carinae, in the branching respiratory tree are often a focal point for deposition of larger diameter EPs.

EP removal from the lungs is primarily affected by the cilia lining the non-gas-exchange regions (conducting airways) of the lungs. The cilia push the mucus produced in the lungs, along with any EPs trapped in the mucus, out of the lungs and into the gastrointestinal tract in a matter of hours or days. Finally, roaming macrophages in the gas exchange regions ingest EPs deposited there for removal through the lymph system and may recruit other macrophages into the region. Human macrophages are approximately 17 µm in diameter and can only ingest particles smaller than they are. This causes frustrated phagocytosis, resulting in the release of inflammatory cytokines and other chemicals into the lungs [Barlow et al. 2017; Blake et al. 1997; Padmore 2017; Stayner et al. 2008; Turkevich et al. 2014], including the production of cells with double nuclei [Ishida 2019]. This and other cellular interactions with long lasting biodurable EPs appear to trigger collagen buildup in the lungs, known as fibrosis, and, over a longer period, can produce cancer as well.

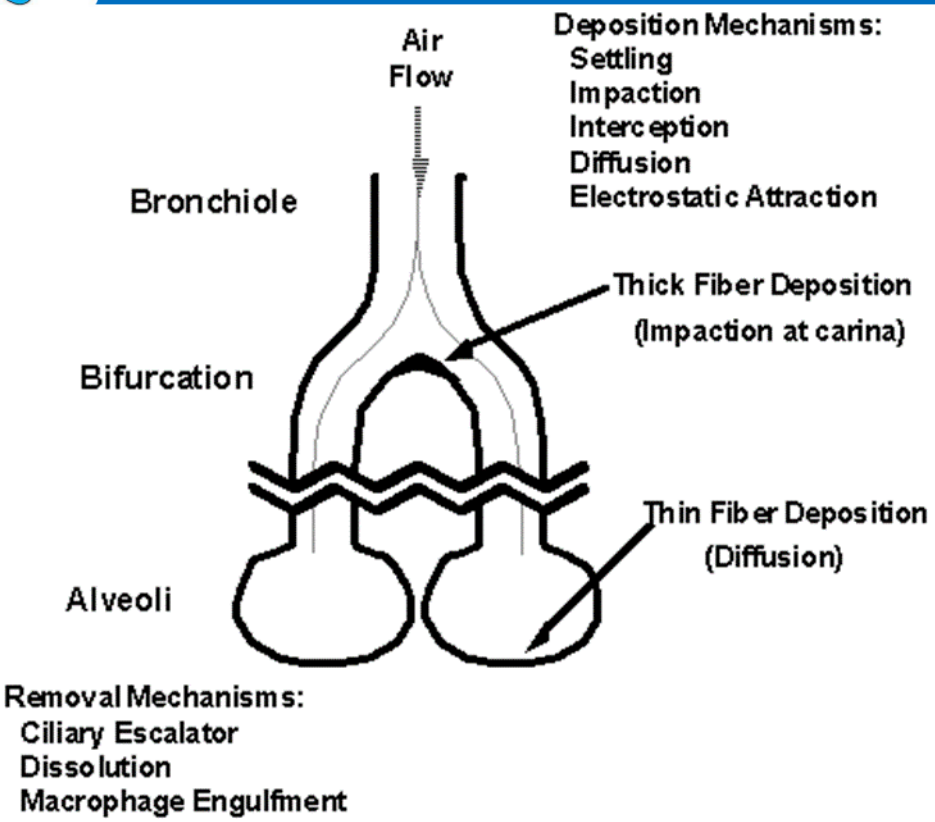

Figure 1. Schematic of mechanisms that affect EP deposition and retention in the lungs.

The surface properties of EPs are also thought to have an effect on toxicity [Groppo et al. 2005; Occella and Maddalan 1963]. The reason being that it can be directly associated with the number of available reactive sites on the EP's surface. Exposed ions on the surface can easily react with cell membranes, causing membranolysis [Fantauzzi et al. 2010]. EPs with low biodurability that dissolve in lung fluid in a matter of weeks or months, such as certain glass EPs, appear to be somewhat less toxic than more insoluble EPs [NIOSH 2011].

f. Exposure evaluation

Asbestos exposure assessment techniques have been in existence since the 1930s [Dement et al. 1983], and some additional techniques began to be used in the 1960s [Rajhans and Sullivan 1981]. Earlier than this, it was not widely recognized that the fibrous nature of asbestos was intimately related to its toxicity, so many techniques involved collection of airborne particles and counting all large particles at low magnification by optical microscopy. Thermal precipitators, impactors (konimeters), impingers, and electrostatic precipitators were all used to sample asbestos. The primary technique in the United States (U.S.) and the United Kingdom (UK) during this early period was the liquid impinger. The liquid impinger impacted an air stream from ambient air at 2.7 liters per minute (L/min) into a liquid reservoir that collected dust particles larger than about 1-µm aerodynamic diameter [Rajhans and Sullivan

1981]. After sampling, an aliquot of the liquid was placed on a slide in a special cell, particles larger than 5-µm size were counted, and the results were reported in millions of particles per cubic foot. Dissatisfaction with this approach stemmed from the lack of correlation between the measured particle concentration and disease in the workplace [Lippmann 1988].

3 Fiber dimension considerations

EPs are often characterized or counted using microscopy methods according to their aspect ratio, i.e., the ratio of the large dimension to one of the small dimensions. The distribution of EP dimensions in a sample can usually be characterized by assuming a cylindrical geometry (i.e., the two small dimensions are identical) and measuring the length and diameter of an individual EP. The distribution of airborne EP sizes generated by grinding bulk material or by mechanically releasing particles into the air often results in a two-dimensional (bivariate) lognormal distribution. Such a distribution is characterized by five parameters: the geometric mean length, the geometric mean diameter, the length and diameter geometric standard deviations, and a correlation term that relates length to diameter [Schneider et al. 1983]. In addition, several other parameters that are a function of length and diameter, such as aerodynamic diameter, can also be characterized by a lognormal distribution [Cheng 1986].

Often the discussion of EPs assumes they are straight cylindrical objects that can be well defined by idealized geometry parameters. However, many real-world EPs are not so simple to describe. EPs can be contaminated by the attachment of other dust particles, creating a complex structure with aerodynamic behavior not matching that of cylindrical EPs. The complexity of EP shapes affects all of the measurement and separation techniques described below, frequently making it difficult to compare one method to another; for example, ISO and ASTM mass determination methods can use square shapes for amphiboles.

For EMPs, their detailed features can aid in their identification. For example, asbestos EMPs are often curved, have splayed ends, or differ in other ways from a cylindrical shape (the majority of asbestos is chrysotile and a cylinder, which is an approximation that can be used for mass estimation for other types) [Champness et al. 1976; Langer et al. 1974; McCrone 1980; Spurny et al. 1979]. An asbestos mineral is composed of fibrils [NIOSH 2011] (about 0.03-µm diameter) that are packed together. This fibrillar structure is characteristic of asbestiform minerals. When the mineral is broken apart mechanically, the material separates primarily on the major axis between fibrils, and the resulting fibers are usually narrower bundles of fibrils. The ends of these narrower bundles can be further broken apart, with smaller individual fibrils spread apart yet still be part of the bundle.

There are many types of EP materials being produced for commercial purposes. These include fibrous glass, mineral wool, refractory ceramic fibers, wood and other plant fibers, and synthetic organic fibers. Most of the fibers of these materials generally have larger diameters than asbestos fibers. On the other hand, CNTs are very thin (<0.005-µm

diameter), and because of their high tensile strength, high conductivity, and other special properties, show great promise as a commercial material [Liu et al. 1998; DeVolder et al. 2013]. The complexity and variety of structures make CNT fiber counting a challenge. Measurement techniques must be tailored to the size distribution and physicochemical properties of these commercial materials.

4 Phase contrast light microscope counting (PCM)

As asbestos-induced disease became widely studied in the 1960s, cellulose-based membrane filter sampling was applied to asbestos sampling in combination with high magnification phase contrast light microscopy (PCM) for counting EMPs. This technique involves air sampling, resulting in a relatively uniform distribution of particles, EPs, and EMPs over the surface of a cellulose ester filter. The filter or a segment of the filter is then placed on a microscope slide, made transparent, and the particles observed on the filter with a high magnification (~450X) phase contrast light microscope. Over the years, many researchers have tried to improve and standardize the PCM method. One researcher, W.H. Walton, discussed many aspects of this technique in a review [Walton 1982]. The high variability of the analysis results and the method's dependence on operator technique made method improvement and research difficult. The PCM method does not typically measure all asbestos EMPs; only those approximately >0.25-µm diameter are visible and only those >5-µm length are counted by protocol. Therefore, the PCM method is only an index of exposure and uses the assumption that what is detected is correlated with the EMPs actually causing disease [Lippman 1988].

The PCM method does not allow differentiation of asbestos EMPs. This is an important limitation when the method is used in settings where EP concentrations with a substantial nonasbestos fraction may occur. This should be remembered when considering some of the parameters discussed next. The aim of evaluating changes to the PCM technique may depend on whether consistency with other laboratories within a country or throughout the world is more important than making measurements that are more closely related to health effects. Several PCM EP counting methods have been published by national [HSE 1990, 2006; NHMRC 1976; OSHA 1998] and international organizations [ASTM 2006; ISO 2014; WHO 1997]. Most countries have methods similar to those referenced here. A number of factors which influence analysis results have been investigated, including the following:

a . Microscope-related parameters

1.) **Microscope magnification**

The exact level of microscope magnification depends on microscope design, with most current methods using 450X (±10%) total magnification. Pang and coworkers investigated using 1250X magnification to improve EP detectability, but this has not been adopted in any established methods [Pang et al. 1989]. Pang also investigated the effect of using lower magnification (400X) and found that counts were lower for chrysotile asbestos by 25%, but amosite EMP counts were unaffected [Pang 2000].

2.) **Phase contrast optics**

This contrast enhancement technique allows detection of asbestos EMPs down to about 0.25-µm diameter for chrysotile and about 0.15 µm for amphiboles. Other techniques such as dark field microscopy may offer improved detectability but also increase the background from nonfibrous particles.

3.) **Test slide to check optics**

A test slide was developed to allow a check of proper alignment and magnification in the microscope [LeGuen et al. 1984]. This ensures a reasonable level of uniformity in microscope setup and operation, including the operator's visual perception. Improper setup can reduce detectability of EMPs. There have also been cases where the optics were "too good," and results were obtained that were higher than the reference count.

4.) **Counting area in microscope field**

Some early measurements with the phase contrast microscope were made using a rectangular graticule for defining the counting area, while others were made using the entire microscope viewing area. It was found that larger viewing areas resulted in lower counts, so the Walton-Beckett graticule [Walton and Beckett 1977] was developed, which nominally gave a 100-µm diameter counting area (the area is calibrated precisely for each microscope) and has been incorporated in all current methods.

b . Sample preparation techniques

1.) **Filter type**

Virtually all measurements are made using 0.8-µm pore size mixed cellulose ester (MCE) filters. Some measurements are made using 1.2-µm pore size filters when sampling low concentrations to allow a higher flow rate through the filter. Smaller pore size filters are used to ensure that EPs are deposited as near the surface of the filters as possible. This results in EPs ending up in the same plane so that they can be readily viewed with a minimum change of focus during EP counting. Pore sizes smaller than 0.8 µm are only used with line-operated pumps because of limited suction power available with personal sampling pumps.

2.) **Selection of the liquid for making filter transparent**

A liquid is placed on the filter that closely matches the filter refractive index yet has an index that is as far as possible from that of the EMPs being detected. Rooker et al. [1982] showed that the refractive index difference between a cleared filter and EMPs translated directly into greater detectability of small diameter EMPs. A viscous solution of dimethyl phthalate and diethyl oxalate mixed with cellulose filter material was commonly used in the 1970s and early 1980s. However, it did not result in a

permanent sample, with crystallization of the mount and movement of EPs often occurring several days after sample preparation. Permanent slides were needed for quality assurance purposes, and the sample preparation technique was also slow and required some skill. A rapid acetone-based filter clearing technique was developed that could be used safely in field situations [Baron and Pickford 1986]. After clearing, filters were coated with triacetin to surround the EPs. This resulted in a longer lasting sample (typically months to years) and is the technique currently specified in most methods. Another technique uses a resin called Euparal to surround the EPs, resulting in a permanent slide preparation [Ogden et al. 1986]. This method has been adopted in the NIOSH [2019] PCM method.

3.) **Filter loading**

The number of EPs on a filter is usually specified to be within a certain loading range to ensure consistent counting. Cherrie et al. [1986] demonstrated using a serial dilution technique where counting efficiency was a function of the concentration of EPs on the filter. At very low filter loadings, i.e., <100 EPs per square millimeter (mm^2), there was a tendency to count high relative to an intermediate range of concentrations (100–1300 EPs/mm^2), where the counts were a linear function of loading. This "overcounting" was apparently due to greater visibility of EPs in a clean visual field. This effect was noted for both human counters and an image analysis system. At high filter loadings (>1300 EPs/mm^2), undercounting occurred due to the overlap of EPs with other EPs and with nonfibrous particles. Most published methods indicate that optimum counting occurs within the 100–1300 fibers/mm^2 range, while some restrict the range further to less than 650 fibers/mm^2.

4.) **Fiber counting rules**

The EP counting rules for most current optical methods indicate that a countable EP should be longer than 5 µm, narrower than 3 µm, and have an aspect ratio greater than 3:1. These rules were selected because shorter EPs were difficult to detect by optical microscopy, and the 3:1 aspect ratio was used to discriminate between fibrous and nonfibrous particles in occupational settings. There has been a great deal of controversy over these rules. The use of a longer EP cutoff, e.g., 15–20 µm, has been suggested, based on two separate arguments. First, that most asbestos EMPs are relatively long and thin (with high aspect ratio), and the longer EP cutoff would discriminate better toward EPs that were truly asbestos EMPs according to mineralogical definitions [Wylie 1979]. Second, that EPs that enter the lungs are removed readily by macrophages if they are shorter than about 15 µm [Blake et al. 1997]. Longer EPs cannot readily be engulfed by macrophages, thus staying in the lungs for a long period, causing continuing fibrosis. However, it is possible that high doses of short fibers could be sufficient enough that the macrophages cannot efficiently remove them through

natural clearance mechanisms, and this could lead to an enhancement of fibrosis.

The aspect ratio criterion has also been questioned because many nonasbestiform particles have length and width distributions that include EPs with aspect ratios greater than 3:1. Because asbestos and other minerals often contain particles, cleavage fragments, and EMPs not in the asbestiform habit, it has been argued that these should not be counted. However, the Occupational Safety and Health Administration (OSHA) has supported the 3:1 minimum aspect ratio through legal precedent. The National Institute for Occupational Safety and Health (NIOSH) has noted that because of the great difficulty in differentiating whether individual high aspect ratio particles are cleavage fragments or asbestiform fibers, all such particles should be counted. These high aspect ratio particles may cause disease whether or not they are asbestiform. As stated previously, the method provides an "index" of exposure that is useful in determining occupational exposure [Lippman 1988]. See NIOSH REL in the Introduction Terminology Section earlier in this chapter [NIOSH 2011].

Other aspects of EP counting have been investigated, including how to count nonstandard EP shapes, overlapping EPs, overlapping compact particles on EPs, and bundles of EPs. Each of these factors can have a noticeable effect on the final count. Cowie and Crawford [1982] investigated the effect of some of these factors and estimated most of them made a difference in the final count on the order of 20%. Many of the methods currently in use have slight variations in their interpretation of which EPs to count and thus can contribute to variation in results between countries and organizations.

NIOSH Method 7400 contains two sets of counting rules: the A and the B rules. The A rules are used for asbestos and are consistent with counting rules in previous NIOSH methods [NIOSH 2019]. The A rules conform to the OSHA 3:1 aspect ratio rule and do not have an upper diameter limit for which EPs are counted. The B rules were introduced as an alternative to the A rules when Cowie and Crawford [1982] found that they provide better reproducibility between laboratories. The B rules are offered by some commercial laboratories for use with EPs other than asbestos for informational purposes because the B rules have 3 µm as an upper diameter limit. This upper diameter limit substantially reduces the counting of typically large-diameter EPs, e.g., glass and cellulose, which are unlikely to deposit in the lungs [Breysse et al. 1999].

c. Quality assurance approaches

1.) **Sample recounts**

Most methods require individual counters to recount about 10% of the field samples to ensure consistent counting procedures and alert the analyst in the case of problem samples. It is also recommended that counters have samples that are routinely recounted to ensure consistent counting within a laboratory over time [NIOSH 2019].

One of the difficulties in analyzing errors made by analysts during PCM counting is that individual fields are difficult to relocate after the analyst has finished counting a slide. Differences in counts between analysts have often been ascribed to local variations in loading on the filter. Pang's development of a slide coverslip that defines counting areas on the sample solves this problem [Harper et al. 2009; Pang 2000]. Areas on the coverslip are vacuum coated with a thin layer of gold and platinum, using an electron microscope grid as a mask. This leaves defined areas on the coverslip that can be located by grid index marks. Thus, specific fields in a sample can be readily located. Using this grid mapping approach, the location, orientation and shape of each EP can be noted, and differences in counts can be reconciled on an EP-by-EP basis. The coverslips have been used to study EP counting accuracy by comparing routine counting of specified fields to counts agreed upon by a group of competent counters. It was found that the principal differences for chrysotile EMP sample counts were due to missing EMPs close to the visibility limit, while the principal differences for amosite EMP samples were caused by incorrectly sizing of EMP length near the 5-µm limit. The chrysotile samples were therefore typically undercounted (negative bias), while the amosite samples had increased variability with individual counters being biased either high or low. Both these errors can be reduced by training counters with pre-counted reference slides prepared using Pang's coverslips [Pang 2000]. In addition, these reference slides can be used on a routine basis to ensure consistency in counting. Pang's coverslips or modified versions show great promise for training analysts and perhaps for improving quality assurance schemes [Harper et al. 2009].

2.) **Interlaboratory sample exchanges**

Crawford et al. [1982] found that using sample exchange programs was more important in ensuring agreement between laboratories than similarity in details of the counting rules. Thus, the exchange of field samples between laboratories is commonly performed to improve reproducibility of counting. A description of several quality assurance techniques for asbestos EMP counting is described by Abell et al. [1989]. To fulfill NIOSH Method 7400 requirements for an interlaboratory sample exchange, Tombes and Calpin [2002] described a simple approach using appropriate statistical tests.

3.) **Quality check samples**

In order to get agreement between laboratories within a country or

internationally, several programs send out identical samples to participating laboratories to assess their relative performance [Arroyo and Rojo 1998; Crawford et al. 1992; Harper et al. 2009; Kauffer 1989; Schlecht and Shulman 1986]. These programs provide valuable data that allows laboratories to ensure that their results are similar to that of other laboratories using reference samples.

d. Qualitative fiber analysis (optical)

The use of identification techniques is not allowed in reporting EP counts using NIOSH Method 7400 to ensure that laboratories are using the same methods for counting and eliminates a source of analyst subjectivity. Considerable confusion has been caused by individual laboratories using qualitative identification techniques to change the counting procedure and, hence, the final results. Despite this, work has been done to find simple rules based on EP dimensions to be able to differentiate asbestiform from nonasbestiform EPs with some success using reference standards [Harper et al. 2008]. Other techniques are available for providing at least tentative identification of EP type—use of these techniques is commonly called *differential counting*, which uses polarized light to distinguish some nonasbestos EPs. Polarized light techniques are based on the optical properties of the materials, including refractive index and crystallinity. These techniques can provide reasonably certain positive identification for EMPs.

EP shape criteria can also be used to differentiate EP types of interest. Glass fibers tend to be straighter, with smoother sides than chrysotile EMPs, for instance. These techniques are often used in the analysis of bulk materials [NIOSH 1994a]. If differential counting is used in reporting a PCM result, any alternative method or modifications to NIOSH Method 7400 that were used in developing the count should be reported to avoid any confusion.

e. Sampling volume for asbestos abatement applications

Sampling for asbestos after abatement requires the selection of a sampling volume that produces high confidence that the air concentration meets standards. The following approach is an example of how to calculate the sampling volume needed to produce a high level of confidence that a target exposure standard (e.g., NIOSH REL, OSHA PEL, EPA clearance standard) is met.

The U.S. Environmental Protection Agency (EPA) authorizes the use of PCM for some clearance monitoring applications, specifying that a level of 0.01 fibers per milliliter (mL) be met. The term “fibers” is used in this example because the methods are written using the term “fibers.” NIOSH Method 7400 indicates that the limit of detection (LOD) for PCM analysis is 7 fibers/mm^2 based on interlaboratory variability. Under the heading “Evaluation of Method, Interlaboratory Comparability,” NIOSH Method 7400 provides the formulas for calculating the confidence limits on a single analysis result. The interlaboratory variability (see Figure 1 in [NIOSH 2019]) at the LOD (7 fibers/mm^2) is such that the upper 95%

confidence limit on a measured value is 4 times greater than the measured value, which is the basis for using the factor of 4 in the equation below.

The equation in the sampling section in NIOSH Method 7400 [NIOSH 2019] can be used to estimate the sampling volume:

$$time = \frac{4 * LOD\left(\frac{f}{mm^2}\right) * (eff.area\ of\ filter\ mm^2)}{flow\ rate\left(\frac{L}{min}\right) * Limit\left(\frac{f}{cc}\right) * 1000\left(\frac{cc}{L}\right)}$$

Rearranging:

$$sample\ volume = \frac{4 * LOD\left(\frac{f}{mm^2}\right) * (eff.area\ of\ filter\ mm^2)}{Limit\left(\frac{f}{cc}\right) * 1000\left(\frac{cc}{L}\right)}$$

With the appropriate values inserted, the equation becomes

$$sample\ volume = \frac{4 * 7\left(\frac{f}{mm^2}\right) * (385\ mm^2)}{0.01\left(\frac{f}{cc}\right) * 1000\left(\frac{cc}{L}\right)}.$$

Where:

f = fibers
L = liter
LOD = Limit of Detection in fibers per millimeter squared
Limit = the target air concentration in fibers per cubic centimeter (cc)

Solving this equation for sampling volume gives 1,078 L. This is the minimum volume that will give a result, allowing a single sample to indicate compliance with the 0.01 fibers/mL limit with 95% confidence. It requires that the sample gives a result less than or equal to the LOD or 5.5 fibers per 100 fields. A higher fiber count may still indicate that the concentration meets the target level, but not with the same level of confidence. This is likely to be a low estimate of concentration and additionally ensure compliance with the standard because the fiber concentration is low, and low fiber loadings are usually overestimated as described in the Filter Loading Section. However, the background concentration of nonfibrous dust on the filter must also be low to ensure that fibers are not obscured.

5 Polarized light microscopy (PLM) of bulk materials

PLM is often used to determine the asbestos content in representative samples of bulk materials. Not all EPs in a bulk material may be asbestos. PLM allows for the identification of asbestos, which is why PCM is not used. The asbestos EMPs in bulk material can be released and become airborne when the bulk material is disturbed. For this reason, it is desirable to measure the asbestos content of bulk samples. The EPA [EPA 1987a] has defined asbestos-containing material (ACM) as material containing more than 1% asbestos using the PLM method, which effectively estimates concentration by area observed. Some confusion exists regarding the units of the asbestos percentage. EPA originally indicated that the limit for ACM was 1% by mass [EPA 1987a], but because of the difficulties in determining corrections for differences in material density and in determining particle volumes, the limit was changed to 1% by area as determined by the PLM method [EPA 1990c]. OSHA does not specify units for percent asbestos in bulk materials in its regulations [OSHA 1994]. It does state that insulation materials containing greater that 1% asbestos are ACM. However, any amount of asbestos in a material may result in exposures to asbestos in excess of its OSHA permissible exposure limit (PEL) and are regulated even if the amount is less than 1% [Baron 1993].

Several PLM techniques are used for identifying EMP types as well as semi-quantifying the percent fibrous material (usually asbestos) in a sample [EPA 1990a; ASTM 2016; Beard and Rook 2001; ISO 2014; McCrone et al. 1978; Middleton 1979; NIOSH 1994a; OSHA 1995; Perkins and Harvey 1993; Vallero and Beard 2009]. These techniques depend on particle shape, the refractive index, and other optical properties of individual particles. Many of these PLM techniques require visual observation of color in the EP and become less reliable for EPs thinner than about 1 µm [Baron 1993; Vaughan et al. 1981].

a. Sampling

Several procedures have been suggested for obtaining representative bulk samples of ACM that prevent unnecessary exposure to asbestos aerosol [EPA 1985a,b; Jankovic 1985]. Representative sampling of commercial ACM is often problematic as these materials may vary substantially in asbestos concentrations between nearby locations and even at different depths at the same location. Sampling from multiple locations and compositing samples helps improve the likelihood of obtaining a representative sample. Different types of materials will most likely also require different mechanical means of sampling, such as how a hard versus soft material is sampled.

The material should be wetted or sealed during sample removal. A small coring device, such as a cork borer, can be used to obtain a sample from the full depth of the material. At least three samples per 1000 square feet (ft^2) of ACM should be taken [EPA 1987a]. The sample should be placed in a well-sealed, rugged container. Finally,

the sampled area should be repaired or sealed to minimize further EMP release. Surface sampling has been proposed by several groups, but there is no relationship between airborne EMPs and those found on surfaces [Chatfield 2000]. Therefore, surface sampling for EMPs is not recommended.

b. Sample preparation for analysis by PLM

Sample preparation for a PLM analysis involves grinding the material to the optimum particle size range (1–15 µm diameter) and dispersing the particles in a liquid of known refractive index on a glass slide [Perkins and Harvey 1993]. It is important to not over-grind asbestos-containing materials, as over-grinding can lead to the deformation of the morphological features of EMPs and may induce a variable degree of lattice distortion [Langer et al. 1978; Van Orden et al. 2012]. This can result in a lack of crystallinity at the particle surface [Groppo et al. 2005; Occella and Maddalan 1963]. For example, a round-robin evaluation of the CARB 435 preparation procedures demonstrated that "…the finer a rock is ground, the less asbestos is reported by a laboratory (either because the asbestos is too small to be observed or because the grinding destroys the asbestos characteristics" [Van Orden et al. 2012].

Particle size uniformity in the prepared sample is extremely important in obtaining a representative sample. A few large chunks of material, which may not be representative, may contain more or less asbestos than hundreds of much smaller particles. Friable material, which is crumbly or can be crushed by hand, may readily release EMPs and is considered more hazardous. Friable materials are generally easier to prepare for analysis than some other ACMs, such as vinyl asbestos floor tiles, which may require dissolution or ashing of the matrix material so that the EMPs are separated and visible in the microscope. Before and after preparation, the sample is observed with a stereomicroscope at 10X–100X magnification to evaluate sample uniformity and observe whether fibrous material is present.

c. Fiber identification using PLM

EPs are immersed in a fluid selected to have a known refractive index. When particles are placed in a liquid whose dispersion is different from that of the particle, the particle may exhibit a color caused by the refraction of light. This technique requires the use of special "dispersion staining" optics [McCrone 1980]. Also, when an EP has a larger refractive index than the surrounding fluid medium, the bright halo (Becke line) around that EP appears to move into it as the microscope focus is raised; when the EP has a smaller refractive index, the Becke line moves out of it. Placing the fibrous material into several fluids with different refractive indices allows the EP refractive index to be bracketed [Frandsen 2016].

Once the sample has been uniformly dispersed on a slide in the appropriate refractive index liquid, specific EP types, e.g., asbestos, can be identified and the percent asbestos estimated. Two approaches are typically used: visual comparison with prepared reference slides or pictures and point counting. When attempting to estimate whether a material is an ACM (i.e., >1% asbestos), the visual comparison

technique is adequate when more than about 10% of the particles observed are asbestos. Point counting is used for lower concentration samples to provide higher accuracy [EPA 1990b]. It involves observing 400 or more randomly selected "points" (identified with a reticle crosshair) in the sample. The number of points containing asbestos is divided by the total number of points observed to give the percent asbestos. A combination of these approaches balances the analysis time and accuracy of the results [Webber et al. 1990].

EP morphology, i.e., the shape of the EP, can be used to assist in its identification. For instance, chrysotile EMPs tend to be curved or curly, while amphibole EMPs are straight, especially when they are shorter than 50 µm. Asbestos EMPs often have frayed or split ends, while glass or mineral wool EPs are typically straight or slightly curved with fractured or bulbous ends. Many plant EPs are flattened and twisted, with diameters between 5–20 µm. Note that it is not recommended to identify EPs solely based on morphology.

EP refractive index and other crystalline properties can be used to identify EP type with reasonable certainty. Several techniques for determining these properties can be used in a polarized light microscope. When viewed in the microscope with crossed polarizing filters, isotropic (isometric or amorphous) EPs appear consistently bright when rotated, while anisotropic (uni- or biaxial crystal structure) EPs appear bright, but disappear when rotated to their extinction angle, which is a function of crystal structure. Thus, amorphous materials such as glass or mineral wool EPs can easily be discriminated from asbestos EMPs.

Elongation of an anisotropic EP can also be determined while using crossed polarizing filters. An accessory plate with a known slow light vibration direction, such as a quartz plate or a compensator, can be inserted, and changes in the interference colors in the EP are then noted. When the longest dimension of the EP is situated in the same direction as the accessory's direction of vibration, the wavelength of light will change, either increase or decrease. EPs with lower wavelengths of light along the longest dimension of the EP have positive signs of elongation ("length-slow"). EPs with higher wavelengths of light along the longest dimension of the EP have negative signs of elongation ("length-fast").

Additional optical properties can be used to identify EPs, including the color/pleochroism or birefringence of the EP. Pleochroism and birefringence are both characteristics of anisotropic materials. Materials can demonstrate pleochroic effects if the specimen's brightness and/or color changes when the microscope stage is rotated in plane-polarized light. Birefringence is a measure of the difference between the high and low refractive indices of an anisotropic material. The refractive indices are measured or observed quantitatively or qualitatively through observation and interpretation of the dispersion staining colors or observation of the interference color (retardation) relative to the particle's thickness [Delly 2019].

Other materials present on the slide can sometimes hamper EPs' accurate identification. Materials that interfere with identification either by their similarity or by covering up the EPs can be removed by physical treatment of the sample. For instance, organic materials, such as cellulose EPs or diesel soot, can be removed by low temperature oxygen-plasma ashing [Baron and Platek 1990]. Leather EPs and chrysotile have a similar appearance and refractive index. The leather can also be removed by ashing at 400°C [Churchyard and Copeland 1988].

PLM analysis is primarily used for qualitative identification of EP type. Accurate identification of asbestos and other crystalline EPs and EMPs requires proper training in the crystallographic properties of particles, as well as training and familiarization with PLM. As with EP counting, a laboratory quality assurance program is necessary to ensure consistently accurate results. The National Voluntary Laboratory Accreditation Program (NVLAP), operated by the National Institute for Standards and Technology (NIST), inspects laboratories for proper practice and provides reference samples that are unknown to the laboratory four times a year to check their performance in EP identification. Under a predecessor to this program, approximately 350 laboratories correctly classified 98.5% of the samples as asbestos and correctly identified the specific asbestos types in approximately 97% of the samples. A blind test of 51 laboratories resulted in 97.5% correct classifications and 79.1% correct identifications [EPA 1986]. The American Industrial Hygiene Association (AIHA) Proficiency Analytical Testing Program (PAT) provides similar PLM bulk asbestos audit samples to laboratories. Some common interferences for bulk analysis by PLM include sepiolite, vermiculite, and cleavage fragments of nonasbestos amphiboles.

PLM has been cast in a quantitative measurement role by the EPA requirement of determining whether a school building material meets the 1% asbestos level defining ACM. Many variables, including particle size, density, and shape, are not adequately controlled or measured in the analysis and contribute to errors in the percent mass estimate. However, because the regulatory requirement has only one significant figure, with careful analysis and certification requirements, it is adequate.

6 Electron microscopy

Scanning electron microscopy (SEM) has not been the focus of as much method development as either light microscopy or transmission electron microscopy (TEM) in asbestos EMP identification. PCM found favor because of the low equipment cost and lower training level required for analysis when there is certainty that asbestos is present. When there is uncertainty, TEM analysis should be used. TEM is also preferred for environmental and research studies because it offers the highest resolution and the most robust identification capabilities. TEM allows visibility of asbestos EMPs as small as individual fibrils, electron diffraction for crystal structure identification, and energy dispersive X-ray analysis for elemental measurement. SEM typically has intermediate resolution, with many instruments of this type not able to see all asbestos fibers.

However, many modern SEMs have the capability of detecting asbestos fibrils. Energy dispersive X-ray analysis is also available for many SEMs, providing some qualitative information of EP type. However, since electron diffraction typically cannot be performed by SEM, particle mineralogy cannot be identified.

a. Scanning electron microscopy (SEM)

Particles are observed in the SEM when a beam of electrons is focused onto the sample surface and scanned over an area. Some of the electrons are scattered from the surface and detected above the surface synchronously with the beam scan rate, and an image of the scanned surface is created. Thus, the SEM detects the surface particles on a substrate. The best image can be obtained on conducting objects deposited on a smooth, conducting substrate. Particles are often deposited on aluminum or carbon planchets (disks) that fit directly into the SEM or onto polycarbonate membrane (track-etched) filters. The samples are usually coated with gold or carbon to increase conductivity.

Some SEM methods have been developed for EP counting [ASTM 1996; ISO 2002, 2019c; WHO 1985]. These methods are primarily used for inorganic, man-made EPs that have larger diameter EPs than can occur with asbestos. They can also be used for asbestos identification using X-ray analysis. This identification is not as definitive as the TEM methods because of the lack of electron diffraction analysis.

b. Transmission electron microscopy (TEM)

The transmission electron microscope (TEM) allows detection of particle morphology, elemental composition using energy dispersive X-ray analysis (EDX), and electron diffraction analysis down to the smallest asbestos EMPs. Although TEM analysis is potentially very powerful and accurate, the process of sample collection, preparation, and details involved in sample analysis can degrade the quantitative accuracy of the technique. Several more specialized techniques, such as electron energy loss spectroscopy and secondary ion mass spectrometry, have been used for analyzing particles and can also be applied to identification of EPs [Fletcher et al. 2001; Fletcher et al. 2011].

Airborne EP samples for TEM analysis are typically collected onto a filter, usually a polycarbonate membrane or MCE membrane filter. For the latter filter type, the filter is chemically collapsed to form a smooth upper surface on which collected EPs are trapped. Sometimes the surface is etched using a low temperature asher to expose the EPs collected on or near the surface of the original filter. The filter is coated with a carbon film that entraps EPs exposed on the filter surface and the filter material is then dissolved away. The carbon film is transferred to a TEM grid (usually 3-mm diameter) and the sample can be placed in the TEM for analysis.

For NIOSH Method 7402 [NIOSH 1994b], the surface is not ashed because some EPs, e.g., cellulose, may be removed and give an inaccurate total EP count [Baron and

Platek 1990]. Ashing can thus affect the measurement of the asbestos EMP fraction when used in conjunction with NIOSH 7400 [NIOSH 2019].

The above approach to preparing MCE filters for TEM analysis is called the direct-transfer approach, since EPs are transferred to the carbon film with minimum disturbance to the way they were collected. An alternative technique is to dissolve the entire filter in liquid, ultrasonicate the suspension to disperse the particles, and deposit an aliquot of the particle suspension onto a polycarbonate filter for final transfer to the carbon film. This is called the indirect-transfer technique. With the indirect technique, the optimum particle loading of the TEM sample can be obtained and soluble particles can be removed from the sample. However, the suspension process can change the apparent size distribution of the particles and EPs by breaking apart agglomerates or even breaking apart asbestos EMPs into smaller EMPs or fibrils [Sahle and Laszlo 1996]. The breakup problem can be especially severe for chrysotile, causing a large increase in EMP count.

The process of sample collection and preparation is complex and subject to factors that can bias the final result. Because only small portions of the filter are observed during TEM analysis, sampled EPs that deposit nonuniformly onto the filter due to inertial, gravitational, and electrostatic effects will be assessed inaccurately [Chen and Baron 1996]. EPs that penetrate the filter surface and are not transferred to the carbon film will be lost. If the filter is incompletely dissolved away from the carbon film, the sample will be difficult to analyze.

Many of the sources of bias and variability noted in sampling and counting by PCM also apply to TEM analysis. EP counting in a TEM can also introduce biases and variability in the final result. There is a tendency to use the high magnification of the TEM to look for the smallest EPs, while ignoring some of the larger ones. Even so, EPs shorter than 0.5 µm tend to be missed because they are difficult to see in the background clutter of the sample [Steel and Small 1985]. Taylor et al. [1984] found that TEM counting gave poorer precision than counting the same sample by PCM and recommended that the fraction of asbestos EMPs counted by TEM be applied to the PCM count as indicated in NIOSH Method 7402. This combined PCM/TEM approach gave better precision than counting by TEM alone.

In addition to morphology, qualitative analysis of EPs by TEM primarily involves two techniques, energy dispersive X-ray analysis and electron diffraction. X-ray analysis produces responses for each of the elements (typically atomic number >6 but is instrument dependent) present in a particle; the responses occur as peaks in an energy spectrum. Specific asbestos EMPs can be identified using peak intensity ratios observed in standard samples and as specified in the method.

The crystal structure of individual EPs is evaluated using electron diffraction. Typically, selected area electron diffraction (SAED) is used to select a single EP to produce a diffraction pattern consisting of a number of spots. The spot locations

depend not only on the EP crystal structure, but also on the geometry of the electron beam optics and other instrumental parameters. The diffraction spot locations relative to one another give a very specific identification of crystal structure. For easily recognized minerals, such as chrysotile, the visual identification of the diffraction pattern is often sufficient. However, to identify fibers not fitting the electron diffraction analysis pattern for standard asbestos EMPs, careful measurement, or indexing, of the diffraction spots is important.

The combination of X-ray analysis and electron diffraction gives a highly definitive identification of specific minerals. However, as with any analytical methods, there are exceptions that require greater expertise to recognize potential interferences. Some minerals that are difficult to differentiate from regulated asbestos minerals include nonregulated amphiboles such as those occurring in a nonasbestiform habit. Sometimes, these can be distinguished by the shape in the TEM image [Bailey et al. 2004; Belluso et al. 2017]. Amphiboles in fibrous talcs can be intermixed with a crystallographic relationship [Virta 1985]. Careful TEM analysis was shown by Virta [1985] to identify them. It should be noted that the NIOSH REL quoted earlier in the introduction [NIOSH 2011] specifically includes the counting of nonasbestiform varieties of regulated asbestiforms, so distinguishing regulated asbestiforms from nonasbestiforms is not performed in NIOSH methods.

Quality assurance is especially important with TEM analysis of EPs. The NVLAP program provides quality assurance accreditation for laboratories performing TEM analysis using the Environmental Protection Agency's Asbestos Hazard Emergency Response Act (AHERA) [EPA 1987a] method. Note that data provided under the AHERA method cannot be directly compared with counts by NIOSH TEM Method 7402 because of significant differences in counting rules, the types of structures counted as asbestos, and the size range of counted EMPs. There are several other established methods for analyzing asbestos EMPs by TEM [ASTM 1998; EPA 1987a,b, 1994; ISO 2019a,b; NIOSH 1994b].

7 Optical detection (light scattering)

Two types of light scattering detectors are commonly used for measuring airborne dust concentrations: the optical particle counter (OPC), which detects and counts individual particles, and the photometer (sometimes called a nephelometer), which detects the scattering from all particles in a defined detection volume. A standard OPC can be used to detect asbestos concentrations in a workplace where the aerosol is primarily fibrous and good correlation with EP counts can be obtained [Rickards 1978]. A nephelometer may also be used but may have an even greater interference from nonfibrous dusts.

A general fibrous aerosol monitor uses an electrostatic alignment technique by applying a field that aligns and rotates individual EPs in a laser beam. The light scattering from the EPs uniquely identifies the presence of individual EPs. This allows the specific detection of EPs [Lilienfeld et al. 1979] and can be used to measure EP length

[Marijnissen et al. 1996]. Several field tests have indicated fibrous aerosol monitor designs agree reasonably well with field measurements of EPs by phase contrast microscopy, though mostly at concentrations above ambient levels. They have been used at abatement sites to provide rapid feedback and ensure acceptable containment of airborne EPs during asbestos removal. New technologies are being developed for asbestos identification in real time [Renard et al. 2020]. One commercially available asbestos aerosol monitor exploits the paramagnetic properties of asbestos along with light scattering to detect all regulated asbestos species in real time and reject nonasbestos EPs with a 99% confidence level [Stopford et al. 2013]. Comparison with standard asbestos methods at abatement sites has demonstrated very good correlation between results.

8 Fiber separation methods

Fiber diameter is known to be a dominant aerodynamic factor. Esmen et al. [1979] showed that the average EP concentration in workplaces decreased exponentially with an increase of EP diameter, indicating that the larger diameter EPs settled out more quickly than smaller diameter EPs. This is due to the aerodynamic diameter of EPs being dependent primarily on EP physical diameter and EP density, with a minor dependence on EP length [Baron 1996]. Baron showed that sampling EPs with a thoracic sampler was approximately equivalent to counting only EMPs with a physical diameter smaller than 3 µm [Baron 1996]. Jones et al. [2001] reported that there appeared to be no impediment to using a thoracic sampler for EP sampling. They found that several samplers matched the thoracic convention, the sample collected by these samplers could be analyzed by standard methods, and that field studies indicated equivalence to the current method [Jones et al. 2001]. Maynard [1999] also found that there appeared to be no variation in penetration through these samplers as a function of EP length.

A spiral centrifuge can be used to separate EPs and reference spherical particles to estimate EP aerodynamic diameter [Stöber 1972]. It was found that the aerodynamic diameter was directly proportional to physical diameter, proportional to the square root of the EP density, and proportional to EP length to the 1/6th power. For EMPs having a density of about 3 grams per cubic centimeter (g/cm^3), the aerodynamic diameter was approximately three to five times the physical diameter of the EP. Behavior of glass EPs in a cascade impactor was investigated by Burke and Esmen [1978]. A small correction to the aerodynamic diameter was developed to take into account the interception of longer EPs with the impaction surface [Feigley et al. 1992]. An inertial spectrometer was used to measure EP aerodynamic diameter and good diameter separation was achieved [Baron et al. 1994a; Morigi et al. 1999]. Baron et al. [1994b] and Deye et al. [1999] developed a technique for separating EPs by length using dielectrophoresis. This technique was also shown to be useful for measuring EP length and diameter distributions [Baron et al. 2000]. It has been shown that aerodynamic size separation of EP aerosols can be accomplished with commercially available equipment using an aerodynamic aerosol classifier and a multi-cyclone sampling array [Lee et al. 2020]. Cyclones, impactors, and porous foam classifiers have been evaluated for efficiency of

removing airborne EPs not likely to deposit in the lungs from a sampling stream [Maynard 1996]. Long EPs can also be effectively removed from an air flow stream by using a screen configuration method [Ku et al. 2014].

Although current U.S. practice does not use an upper diameter limit for asbestos EMPs, a limit of <3 μm is commonly used for man-made EPs outside the United States. It would be advantageous for thoracic sampling to become routine for measurement of asbestos and other EPs. This approach has several advantages. It aligns the analytical method for EPs with conventional practice for dust sampling and counting based on thoracic sampling. It removes some of the larger particles in the sample, resulting in a cleaner sample for the analyst. It removes the need for imposing an EP diameter limit during counting and would align the analytical methods for asbestos and other EPs to those used for all dusts in the U.S. and in the global arena. Thoracic sampling has the disadvantage of requiring the flow rate for a specific sampler to be fixed. This reduces the flexibility to target the loading of the filter by adjusting the flow rate. However, several classifiers can be designed to operate at selected flow rates to allow some flexibility in sampling.

9 Other techniques

a. Counting

EP counting by an analyst can produce relatively high biases and variability even among the best analysts and even among a single analyst depending on factors such as time of day or how long they have been on the microscope. Therefore, several researchers have attempted to develop automated counting systems. With the increases in computing power over the last 45 years, significant efforts have been made to develop such a system. An early attempt was made by researchers at Manchester University, in collaboration with the Health and Safety Executive in the UK [Kenny 1984]. They carried out a comprehensive study to develop an EP counting system. The Manchester Asbestos Program (MAP) was able to give reasonably good agreement with manual counting for certain types of samples. It was used as a reference analyst for the U.S. and UK reference sample programs for several years. Eventually, MAP was dropped as the reference because it was not sufficiently consistent for all types of samples.

Image analysis of asbestos EMPs have the following principal challenges: the complexity of many EMP shapes, including bundles, agglomerates, and split EMPs; the EMPs often go in and out of the plane of focus; the background includes many particles and other nonfibrous shapes; the phase contrast optics produces haloes around particles in the sample that can be detected as EMPs; and finally, and perhaps most importantly, the contrast between the EMPs and background is poor, and many EMPs are near the detection threshold. An evaluation of the MAP program indicated that a significant fraction of the EMPs were misidentified as multiple EMPs, not

detected at all, and groups of compact particles or edges of large particles were detected as EMPs [Baron and Shulman 1987].

Inoue and coworkers [1998] developed image analysis software using a microprocessor-based personal computer. Initial tests indicated that it works approximately as well as human counters. Inoue also evaluated how well human counters and the image analyzer did in detecting the same EPs in a sample. They found that only about 50% of the EPs were consistently counted by all counters, so the image analysis system did approximately as well as the manual counting [Inoue et al. 1999]. More recently, Alexandrov et al. [2015] used fluorescence microscopy for improved EP detection before automated counting. This did result in better EP/background separation, but the automated counting routine used still needed operator intervention to obtain accurate results. Biswas [2020] used a machine-learning segmentation method to obtain a 95% counting accuracy on SEM asbestos EMP images.

In addition to image analysis, optical microscopy can be enhanced using a personal computer to more easily observe the image and to mark and measure fiber dimensions with automatic recording of the EPs counted [Lundgren et al. 1995]. This does not appear to improve the counting accuracy because the analyst still decides which EPs are to be counted.

b. Identification

The current best practice for asbestos EMP identification is stated in [ISO 2019b], "…(TEM is) the only technique capable of unequivocal identification of the majority of individual fibres of asbestos." However, this is qualified by the statement, from the same document, "The method cannot discriminate between individual fibres of asbestos and elongate fragments (cleavage fragments and acicular particles) from nonasbestos analogues of the same amphibole mineral." EP identification using a TEM is accomplished by evaluating EP morphology, chemical composition, and crystal structure using imaging, energy dispersive analysis (EDS), and electron diffraction, respectively. The combination of this analytical analysis, identification evaluation, and manual fiber/structure counting can be expensive and time consuming.

Recent work explores the use of several other techniques such as spectroscopies (Raman, infrared, IR) and SEM electron backscatter diffraction (EBSD) for the identification of asbestos. These techniques have the advantage of minimal sample preparation, efficient identification, and the ability to scan larger areas. The disadvantage for optical methods still remains the optical resolution. Both visible and ultraviolet Raman spectroscopy have been explored [Bard et al. 2004; Petry et al. 2006; Rinaudo et al. 2003; Rinaudo et al. 2004]. Rinaudo et al. [2004] demonstrated that visible Raman could identify all asbestos species when the fibers are in bundles of at least 1.5 µm in diameter. Petry et al. [2006] used ultraviolet (UV) Raman and

obtained unambiguous identification of all regulated asbestos types. UV has the advantage in that it does not produce fluorescence, which allows the assessment of hydroxyl-stretching vibrations. However, as in Rinaudo, Petry's data came from clusters or bundles of EMPs that produce high signal to noise ratios.

Fourier Transform Infrared Spectroscopy (FTIR) is another vibrational spectroscopy that has been demonstrated in both the OH-stretching and in the lattice modes regions and can be used for efficient identification of amphibole asbestos types [Ventura et al. 2018]. As in the case of Raman spectra, the analysis was done on large clusters and bundles of EMPs. In the case of EBSD [Bandli and Gunter 2014], transmitted EBSD patterns were used in an SEM. This is in effect a diffraction pattern analysis method, subject to similar complications as for the identification of amphiboles from SAED patterns obtained by TEM. Nonetheless, the authors demonstrated that this technique can distinguish between asbestos phases and other interfering mineral phases. It also has an advantage of being able to scan larger areas than a TEM with the ability to do direct analysis without having to prepare samples utilizing direct transfer or indirect transfer methods. All of these techniques have produced encouraging results but have not been tested on field samples.

In the case of spectral methods, spectra of minerals in the sample can interfere with the desired spectra, however, in some cases, data processing techniques may be able to resolve this. These techniques have not been evaluated with smaller clusters or bundles of EMPs, whereby the signal to noise ratios may be too low, hindering utility. However, because these techniques use either optical microscopes or SEM, they can, in principle, be combined with automated stages and machine-learning counting methods to boost efficiency of analysis even further. In addition, the utility of spectroscopic identification has been improved using machine-learning methods that allow unidentified spectra to be matched to known spectra in a large database based on probabilities [Zheng et al. 2018]. Bandli and Gunter [2014] suggest this may be possible for EBSD also because some EBSD databases already exist.

10 Conclusions

The capability to measure EP size distributions is available through microscopy and, to a much lesser extent, through direct-reading instrumentation. PCM, PLM, SEM, and TEM methods often do not produce directly comparable results because of differences in counting rules, resolution capability, and the ability to distinguish asbestos from interfering particles or other EPs. The traditional methods of microscopy are relatively inaccurate when compared to chemical analysis methods for most other analytes because of the many sources of error in the sampling and analysis procedure. To improve laboratory-to-laboratory agreement, training, quality control (including the exchange of samples among laboratories), and proficiency testing are important. Implementation of training through the use of Pang's coverslips allows for the investigation of counting errors, and therefore, the potential improvement of PCM

counting accuracy. Thoracic sampling could eliminate interfering particles and thereby improve measurement methods in the future.

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